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Vaginal bacterial microflora modifications during the growth of healthy cows

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C. OTERO, L. SAAVEDRA, C. SILVA DE RUIZ, O. WILDE, A.R. HOLGADO AND M.E. NADER-MACÍAS. 2000. The aim of this work was first, to determine the predominant groups capable of colonizing the vagina and maintaining high numbers with time. The normal microbial flora of the cow's vagina and its evolution from weaning to service was then studied using standard microbiological methods. The results show that the most dominant bacteria belong to the streptococci, followed by the staphylococci, with similar levels during the whole study period. Enterobacteriaceae and lactobacilli were present at very low levels, the latter increasing during the cow's growth, suggesting some kind of hormonal influence. The results will allow the selection of micro-organisms with probiotic characteristics, classified as GRAS (Generally Regarded as Safe), to be used in the prevention of infections in the vaginal tract of cows, such as metritis, which produces delayed periods between partum and conception, and consequent economic losses.

INTRODUCTION

The normal microbial flora of the bovine urogenital tract is made up of a dynamic mixture of aerobic, facultative anaerobic and strict anaerobic micro-organisms. In such a lively and dynamic ecosystem, new strains are continually being introduced (Hafez 1993). Under natural conditions, this environment is stable, protecting the host from the onset of pathogenic or potentially pathogenic saprophytic micro-organisms. The normal microbial flora of this tract is composed of bacteria of the genera *Staphylococcus, Streptococcus* and the coliform group (Hafez 1993). The majority of studies on this subject, carried out by Bartolomé *et al.* (1996), relate to the isolation and identification of micro-organisms during the postpartum or puerperium, with special focus on the incidence of decreasing fertility and persistent metritis (White *et al.* 1989; Montes and Pugh 1993; Torres *et al.* 1994).

In a previous paper, the isolation and identification of aerobic micro-organisms from the vaginas of cows during the oestrous cycle was reported (Otero *et al.* 1999). The aim of the present work was to study the qualitative and quantitative changes in the vaginal microflora during the growth of heifers, with special emphasis on the genus *Lactobacillus*.

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MATERIALS AND METHODS

Animals

A total of 15 heifers (Criolla breed) was used in this study. They were fed *ad libitum* and grown and maintained at the Leales Experimental Fields of INTA (National Agricultural and Livestock Technology Institute) in Tucumán, Argentina.

Samples

Ten series of 15 vaginal samples were obtained over an 18 month period while the cows were growing. The first series of vaginal samples was taken at 7 months after the heifers were weaned. The last sample was taken several days before they were serviced. Before taking the samples, the vulvar area was washed with water, disinfected with iodine-povidone and then washed again with water. A disposable speculum was put into the vagina. Two samples from the posterior area of the vagina were taken from each animal with long sterile cotton swabs. They were collected in LAPT (Raibaud *et al.* 1963) broth and LBS (Lactobacilli

Selective Media) broth (Rogosa and Sharpe 1963), and kept on ice until they were brought to the laboratory. The sample weights were between 2·3 and 2·9 g.

Micro-organism isolation and quantification

Quantification of micro-organisms was performed by serial dilutions using 0·1% peptone. Solutions were then plated onto different enriched media (LAPTg and Blood agar for the total number of bacteria) and selective media: LBS (Lactobacilli Selective Media, Hi-media), SF (*Streptococcus faecalis*, Merck, for enterococci), Mannitol Salt (MSA, Merck, for staphylococci) and McConkey agar (Merck, for enterobacteria). The plates were incubated under aerobic conditions for 24–48 h at 37 °C, except for Blood agar and LBS which were incubated in an atmosphere with 5% CO_2. Identification was performed by morphological and phenotypic characteristics using the following biochemical assays: Gram staining, catalase reaction, coagulase, NO_3 reduction, indole production, type of haemolysis, growth in 6·5% NaCl, sodium hypurate hydrolysis, aesculin hydrolysis, growth in SF broth, Voges Proskauer reaction and fermentation patterns of sugars, complemented with an API system (BioMérieux). The β-haemolytic and catalase-negative strains were identified with serological tests using the Slidex Strepto Kit A, B, C, D, F and K (BioMérieux).

Statistics

The results show the mean value ±S.D. of the data obtained from 15 cows. Analysis of variance was used to study the significance of differences between data (*F*-test).

RESULTS

The results obtained from the isolation and quantification of aerobic micro-organisms from the vaginal tract of growing cows are shown in Fig. 1. Numbers were between 10^3 and 10^5 cfu sample^{-1} during the whole study period, with no statistically significant differences between them ($P > 0{\cdot}05$). Enterococci (Fig. 2) and staphylococci (Fig. 3) were predominant, maintaining 10^2–10^4 cfu sample^{-1}, although the prevalence of enterococci was slightly higher than that of staphylococci. Again, there was no statistically significant difference ($P > 0{\cdot}05$).

Enterobacteriaceae (Fig. 4) and lactobacilli (Fig. 5) followed with a lower number of bacteria. They maintained very low levels in the first study period, with 10^0–10^2 cfu sample^{-1} , but lactobacilli increased in numbers during the final study period.

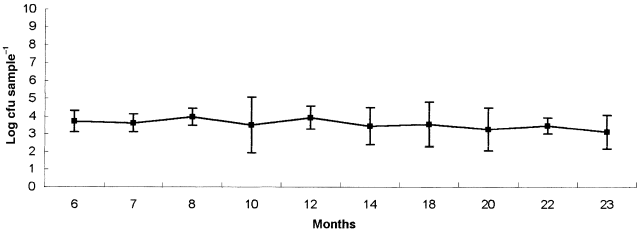

Fig. 1 Quantification of the total number of micro-organisms isolated from the vaginal tract of growing cows. Samples were processed as described in the text. Means ±S.D. of the bacteriological profile obtained from all 15 cows

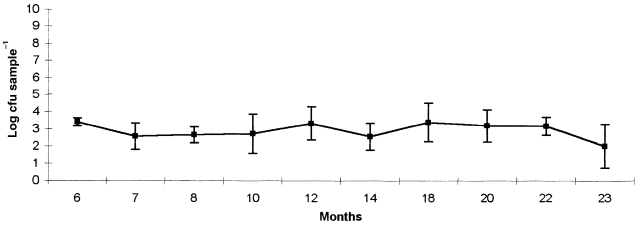

Fig. 2 Quantification of enterococci isolated from the vaginal tract of growing cows

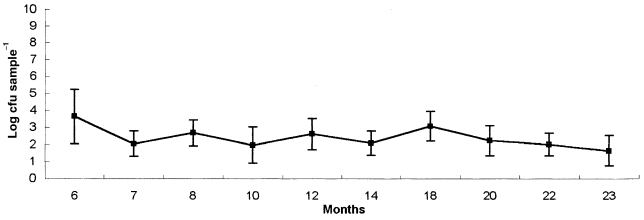

Fig. 3 Quantification of staphylococci isolated from the vaginal tract of growing cows

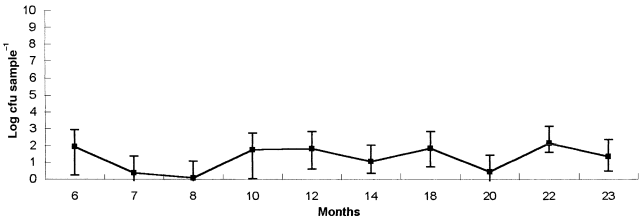

Fig. 4 Quantification of Enterobacteriaceae isolated from the vaginal tract of growing cows

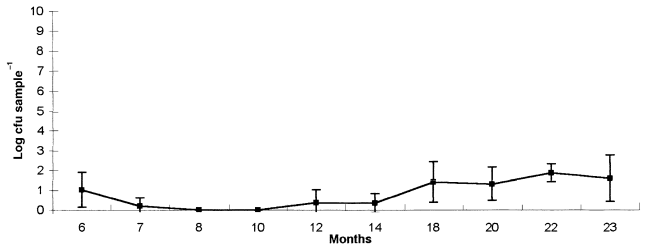

Fig. 5 Quantification of lactobacilli isolated from the vaginal tract of growing cows

DISCUSSION

Productivity of cows is evaluated by the number of calves per year. The interval from calving to conception (ICC) must not exceed 110 days, because the presence on a farm of infertile females significantly increases the production cost (Blanch 1994). One of the main causes of decreased fertility, which lengthens the ICC and decreases the first service conception rate, is puerperal metritis. During the postpartum period, almost 90% of the cows undergo development of non-pathological endometritis, which could be a characteristic of uterine involution. The majority of the animals resolve this clinical situation spontaneously, without affecting reproductive capacity. However, some animals are unable to overcome the problem and suffer severe uterine infections (Lewis 1997).

Conventional intra-uterine treatments with antibacterial substances administered as infusions could appear in milk residues, and do not improve fertility of the animals. Sometimes, they act as irritants, inhibiting the uterine defense mechanisms (mainly chemotaxis and phagocytosis of polymorphonuclear neutrophils) (Campero *et al.* 1992). This is one reason for increasing preventive treatments with natural products which would avoid alterations in milk quality and economic losses due to decreased reproduction. Some of these products are 'probiotics' which have proved beneficial to man and animal. The term 'probiotic' was redefined by Havenaar *et al.* (1992) and Tannock (1999) as "live micro-organisms administered to the host to improve the properties of the indigenous microflora". The probiotic concept has been applied in this work to preparations which could be used intra-vaginally to prevent infections and restore the affected ecological balance. Micro-organisms suggested and used for probiotic purposes are those defined as GRAS, and include the genera *Lactobacillus* and *Bifidobacterium*, and some streptococci (Tannock 1999). One important factor to be considered is that the strains to be used must be isolated from the same host animal as that in which they are going to be applied, and from the same anatomical area, based on the characteristic of host specificity exerted by the species of the indigenous microflora to be colonized (Savage and Kotarsky 1979).

In order to study the probiotic effect in the urogenital tract, an animal model with mice was developed to show the preventive effect of lactobacilli against uropathogenic *E. coli* (Nader de Macias *et al.* 1992, 1996; Silva de Ruiz *et al.* 1993, 1996). Isolation and selection of *Lactobacillus* strains with probiotic characteristics from the vaginal tract of women was also performed (Ocaña *et al.* 1999a,b,c,d). As another interesting aspect is the probiotic effect on the vaginal area of cows, descriptions of the normal microbial flora of the cow vagina were consulted. No description, however, has been reported except for some pathological or physiological situations such as pregnancy (White *et al.* 1989; Campero *et al.* 1992; Montes and Pugh 1993; Torres *et al.* 1994;Amin *et al.* 1996). Previously, the variations of the normal microflora throughout the oestrous cycle were studied (Otero *et al.* 1999) in order to determine the hormonal influence. This paper describes the screening of vaginal micro-organisms during the growth of healthy cows to determine the colonization kinetics and the predominant genera or species. From the results obtained from phenotypic characteristics, the total aerobic microflora represented 10^4-10^5 cfu sample^{-1} during the whole study period. To date, there is no report containing this data in young and healthy cows. Coagulase-negative *Staphylococcus* and α-haemolytic *Streptococcus* contributed to this aerobic flora to a large degree. These genera were also isolated from adult cows' vaginas in large numbers at different times of the reproductive cycle (Amin *et al.* 1996). With reference to coagulase-negative *Staphylococcus*, the predominant species was *Staph. xylosus*, consistent with the findings by White *et al.* (1989).

The number of isolates belonging to the Enterobacteriaceae was lower than that of those belonging to the genera mentioned above. The main species isolated was *E. coli*, consistent with the findings of Torres *et al.* (1994).

The number of lactobacilli was very low, contrary to the results reported for the human, mouse or monkey vagina (Reid *et al.* 1985; Herthelius *et al.* 1989). Their numbers increased in the final study period when hormonal maturation is being completed. The previous report also showed increased levels of lactobacilli during the oestrous cycle (Otero *et al.* 1999). Kummer *et al.* (1997) showed that intra-uterine inoculation of lactobacilli was able to stimulate the cells of the immune system.

The micro-organisms of the genera *Streptococcus and Lactobacillus* isolated and reported in this paper will be included in the selection of those classified as GRAS, according to their probiotic characteristics. They will be

used in the design of veterinary products for the restoration of the normal microbial flora.

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